Ashwagandha [Withania somnifera (L.) Dunal] is an important medicinal plant, has been used for centuries in ancient Hindu system of medicine ‘Ayurveda’ to increase longevity and vitality. It is a subtropical plant of great value, which plays an important role in health improvement around the world. Every part of this plant has been investigated as a source of valuable compounds. The aim of the present study was to assess the phytochemistry, antibacterial activity, nephroprotective property and \textit{in vitro} response of this potential medicinal herb. As this medicinal plant is widely used in several health disorders, it was subjected to intensive research. The earlier reports relevent to objectives of present context are reviewed and presented here.

2.1. Agronomy

Organic farming is ‘farming without chemicals’. It has been practiced since ancient times. The only diversion came when we blindly started using chemicals for agricultural purposes. Our forefathers used all the techniques that now we are reverting back to coming close nature again. So we would not be wrong in saying that we are reinverting traditions or traditional methods (Sofia \textit{et al.}, 2006). Application of biofertilizers and farmyard manure along with inorganic fertilizer are known to support sustainable agriculture in crop and medicinal plant cultivation. Some of the earlier reports on the organic cultivation of \textit{W. somnifera} and other medicinal plants are highlighted here.

In \textit{Avena sativa}, inoculation of \textit{Azospirillum} and \textit{Azotobacter} increased the total N content in the soil at the end of the experiment and increased the nitrogenase activity in the rhizosphere, when compared with N application (Shabaev \textit{et al.}, 1991). Mutualistic association between roots of higher plants (\textit{Prosopis juliflora} and \textit{Zizyphus jujuba}) and VAM fungus helps in establishment and productivity of the plants under experimental stress conditions. The root borne pathogens were suppressed on mycorrhization. Inoculation of VAM was used for the control of rhizosphere fungal pathogens of \textit{Anethuns graveolens} (Joy \textit{et al.}, 2005).
Application of NPK and VAM at the rate of 2 kg/hectare enhanced the availability of soil phosphorus. This might be due to the activity of mycorrhizal fungi or their accompanying microflora by solubilising inorganic forms of phosphorus or by mineralization of organic phosphorus (Kothari et al., 1999). Influence of single and combined application of organic manures and inorganic fertilizers to Mentha arvensis, help in sustaining crop productivity with reduced use of fossil fuel based inorganic fertilizers (Chand et al., 2001). Assessment of the association of VAM fungi on ten important herbs by root infection studies showed that the Glomus was the most abundant VAM fungi associated with all the plants studied (Kalita et al., 2002). Combined inoculation of Azospirillum and Azotobacter was found to increase the chlorophyll content to the tune of 48.5% in Morus alba (Sivakumar et al., 2004).

Studies on the application of various biofertilizers like farm yard manure, vermicompost and combination of Azospirillum and Phosphobacteria to Phyllanthus amarus revealed that the FYM remarkably enhanced the growth of plants in terms of growth of shoot and root length, number of branches, leaves, fruits and dry weight of the plant. Combined application of biofertilizers like Azospirillum and Phosphobacteria with FYM had positive influence (Annamalai et al., 2004). By the combined application of organic manures and fertilizers in W. somnifera, highest yield was obtained and the root yield was increased upto 30 kg/hectare. The plant height, number of branches per plant, root length was also significantly high (Aishwath, 2004). The quality of root alkaloid was found to be better at the low nitrogen level (30 kg/ha) and FYM. However, the root yield was maximum in 20 kg N/ha in W. somnifera (Ajay et al., 2005).

Effect of FYM, vermicompost, poultry manure, Azotobacter and Phosphobacteria was studied on Curculigo orchioides. The combined application supported a yield of 407 kg/ha of dry rhizome with 59.11% starch, 3.04% crude fibre and 11.87% crude protein, 1.71% crude fat, and 11.32 ppm curculigoside (Joy et al., 2005). Application of FYM at 10 t/ha with N, P, K recorded significantly favoured for higher dry tuber yield attributing parameters in Coleus forskohlii (Somnath et al., 2005).
The rhizobacterial inoculation positively influenced the germination, vigour, shoot and root length, biomass and dry matter production, root and alkaloid yield of *W. somnifera*. Inoculation of *Azospirillum lipoferum*, *Azotobacter sp.*, *Bacillus sp.* and *Pseudomonas fluorescence* as combined inoculants, recorded the maximum growth, fruit, seed and alkaloid yield of Ashwagantha (Gopal *et al.*, 2006).

In *W. somnifera*, application of 15 kg N/ha, increased the root length, after 18 days of transplanting (Kaushal and Rana, 2006).

Application of nitrogen 93.75 kg/ha, phosphorus 93.75 kg/ha and *Azospirillum* 2 kg/ha gave highest herbage yield of 17.93 and 19.35 t/ha, and essential oil content 0.189 and 0.210% and oil yield 22.96 and 27.54 kg/ha viz., main and ratoon crop of *Artemisia pallens* (Senthilkumar *et al.*, 2005). Combined treatment of biofertilizers, FYM and NPK was produced the highest yield (386.809 tons/ hectare) in green turmeric *Curcuma longa* (Yamgar *et al.*, 2006). The integrated nutrient combination involving organic form of manures (cocopeat and farm yard manure) and inorganic fertilizers (NPK) showed greater degree of positive influence on the seed yield of *Mucuna pruriens*. The availability of nutrients as well as nutrient uptake was found to be the highest in the same treatment combination (Kavitha and Vadivel, 2006).

### 2.2. Phytochemistry and Pharmacology

*Withania somnifera* is widely used in ayurvedic medicine, the traditional medicinal system of India. Earlier literature pertaining to therapeutic benefits and chemical properties of this herb and some other important herbs were presented here. In this review, previous observations explaining the scientific basis of curing the diseases by various herbal preparations were given more importance.

Presence of sixteen withanolides including three new compounds from aerial parts of *W. somnifera* and their potent of cell differentiation inducing activity against mouse myeloid leukemia cells were studied by Kuroyanagi *et al.* (1999). Antitumor and radio sensitizing effects of alcoholic root extract of *W. somnifera* and their
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modifications by heat were studied in vivo on Sarcoma-180 grown on the dorsum of adult BACB/C mouse. Treatment of ashwagandha increased the effect of radiation on tumor regression as well as growth delay, but ashwagandha treatment and hyperthermia significantly increased the tumor cure and depleted the tumour GSH level (Umadevi et al., 1993). Antitumor and radiosentizing properties of W. somnifera were investigated by Umadevi (1996). The alcoholic extract of the dried roots of the plant and as well as the active component withaferin-A isolated from the extract showed significant antitumour and radiosensitizing effects in experimental mice tumour in vivo and it was proved as a good natural source of a potent and relatively safe radiosensitizer / chemotherapeutic agent.

The aqueous extracts of dried powder of W. somnifera were administered to the male mice with cadmium induced hepatotoxicity and nephrotoxicity. A decrease in lipid peroxidation (LPO) and an increase in superoxide dismutase (SOD) and the catalase were observed in liver and kidney. Intravenous administration of lipopolysaccharide from Klebsiella pneumoniae and peptidoglycon from S. aureus resulted in the elevation of lipid peroxidation in rabbits and mice (Dhuley, 1998). Simultaneous oral administration of aqueous root extract of W. somnifera prevented the lipid peroxidation.

Administration of W. somnifera root extract in the doses of 0.7 gm/kg and 1.4 g/kg body wt. per day along with equivalent doses of lead acetate for 20 days significantly decreased renal and hepatic lipid peroxidation (LPO) and increased the activities of antioxidant enzymes like superoxide dismutase (SOD) and catalase (CAT). Thus retaining normal peroxidative status of the tissues and protected them against lead intoxication and resulted in antiperoxidative action in mice (Chaurasia et al., 2000). Administration of the plant extract of W. somnifera (20 mg/dose/animal i.p.) for five days along with cyclophosphamide (CTX) (1.5 mmol/kg body wt. i.p.) reduced the CTX induced urotoxicity. Treatment of the root extract controlled the inflammation and dark colouration in the bladder and retained the normal morphology of bladder. The extract was found to reduce the protein level in serum and blood urea.
and enhance glutathione (GSH). Histopathological analysis of the CTX intoxicated animal bladder showed severe necrotic damage whereas the *W. somnifera* treated groups showed normal bladder architecture (Davis and Kuttan, 2000).

Matsuda et al. (2001) identified 7 new withanolide glycosides called withanones I to VII from the roots of *W. somnifera* along with withaferin-A, physagulin-D, coagulin-Q etc. Two withanolides namely withacogulin and withatrinolide were isolated from *W. coagulans* by Rahman et al. (2003). Ganzera et al. (2003) made HPLC analysis to find out withaferin-A and withanolide-D in *W. somnifera*. The antioxidant activity of *W. somnifera* glycowithanolides was assessed in chronic foot shock stress. The stress increased the SOD, LPO and decreased CAT and GPX levels in the brain regions. Administration of *W. somnifera* glycowithanolides doses (10, 20 and 50 mg/kg) reverse the stress effects and normalise the augmented SOD, LPO and enhanced the CAT and GPX activities. Thus *W. somnifera* glycowithanolide was lending support to the clinical use of the antistress adaptogen in rats (Bhattacharya et al., 2001).

Glycowithanolides of *W. somnifera* were investigated for their preventive effect on the animal model of tardive dyskinesia (TD) induced by the neuroleptic haloperidol. Glycowithanolides were found superior than antileptic standard allopathic drug sodium valproate in curing this neural disorder. Further, the prevention of haloperidol induced TD was observed due to antioxidant effect of *W. somnifera* (Bhattacharya et al., 2002). Novel withanolide glycosides and withanolides have been isolated from *W. somnifera*, showed inhibition activity against COX-1 and COX-2 enzymes ranging from 9 to 40% and the inhibition effect on COX-2 enzyme was the first report in this kind. It is important to note that over expression of COX-2 enzyme was observed in tumour cells (Jeyaprakasam et al., 2004).Antioxidant, cytoprotective and other relative properties of *W. somnifera* were found out by Geetha et al. (2003). Through in vitro studies it was found to retard the formation of cold cataract and suggesting that ashwagandha as a good cataractostatic agent.
Antistress adaptogenic activity of *W. somnifera* roots was studied using administration of chronic stress induced male rats. The stress induced rats showed symptoms of hyperglycaemia, glucose intolerance, gastric ulcer, male sexual dysfunction, cognitive deficits and mental suppression. The stress induced perturbations were controlled by root extract along with *Panax ginseng*. The results indicate that *W. somnifera* has significant antistress adaptogenic activity confirming the clinical use of the plant in Ayurveda (Battacharya and Muruganandam, 2003). Studies on anti-proliferative and antioxidant activity in human tumorogenic cells, suggested that *W. somnifera* leaf extracts can be used as an antitumour agent but not as an antioxidant substance (Kaur *et al.*, 2004).

Novel withanomides and withanolides were identified from methanolic extract of *W. somnifera* fruits. The isolated compounds inhibited the lipid peroxidation (90%) and had antioxidant activity. The antioxidant activity was expected due to the hydroxylated long chain acyl group (Jayaprakasham *et al.*, 2004). Administration of *W. somnifera* extract in combination with the extract of *Aloe vera* reduced the oxidative damage of brain tissues and declined the lipid peroxidation, protein carbonyl and lowered the blood glucose level of diabetic mice (Parihar *et al.*, 2004).

Ten herbal formulations were administered orally at the dose levels of 125, 250, 500 and 1000 mg/kg in rats suffering from ciplatin induced renal damage. The drug reduced the level of serum creatinine, lactate dehydrogenase, uric acid, blood urea, superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and lipid peroxidation (MDA). It was concluded that the herbal extract possesses antioxidant activity and can protect the heart and kidney from damage caused by ciplatin (Pallavi and Balaraman, 2004). Administration of aqueous fruit extract of *W. coagulans* significantly lowered the blood sugar, serum cholesterol, serum LPO and hepatic LPO levels at the concentration of 1 gm/kg; p.o. in streptozotocin induced diabetic rats. In normal rats also the blood sugar levels were significantly decreased by the above drug. *W. coagulans* also exhibited free radical scavenging activity in an *in vitro* system (Hemalatha *et al.*, 2004). Treatment of immunized (DPT) animals with
plant materials of *W. somnifera* (100 mg/kg/day) for 15 days resulted in significant increase of antibody titres in rats when challenged with pathogen pertussis. It indicates the plant material as potential immunopotentiating agent which can be used in immune chemical industry to reduce the morbidity and mortality of test animals (Gautam *et al*., 2004).

Effect of ethanolic extract of the root of *W. somnifera* against Dalton’s Ascitic Lymphoma has been evaluated in Swiss albino mice. A significant increase in the life span and a decrease in the cancer cell number and tumour weight were noticed in the tumour-induced mice treated with root extracts of *W. somnifera*. The hematological parameters were also corrected by root extract of *W. somnifera* in tumour-induced mice (Christina *et al*., 2004). Commercial ayurvedic formulations showed wide variations in the content of seven medicinally active principles like withaferin-A withanone, withanolide-A, withanolide-D etc. The study made by Sangwan *et al*. (2004) emphasised the need for stringent phytochemical standardization of herbal products. *W. somnifera* root was found with chemopreventive efficacy against forestomach and skin carcinogenesis and warranted the identification and isolation of active compounds responsible for its anticancer effects.

Co-administration of methanolic extract of *W. somnifera* (root) *Ocimum sanctum* (leaf) and *Zingiber officinale* (rhizome) will protect the health disorders in connection to stenuous physical exercise, and on swimming-induced oxidative damage in rats. Stress in rats, resulted in a significant elevation in the level of products of free radicals, and reduced activity of catalase (CAT), superoxide dismutase (SOD), glutathione-S-transferase in testis, prostrate and seminal vesicle which were protected significantly after co-administration of methanolic extract of the said plant parts. This herbal extracts had no toxic effects on metabolic organs that had been proved by the measurement of glutamate oxaloacetate transminase and glutamate pyruvate transminase activities in liver and kidney (Misra *et al*., 2005). Withanolide -A isolated from *W. somnifera* root was used for the therapeutic treatment of neurodegenerative diseases and it was able to reconstruct the neuronal networks (Kuboyama *et al*., 2005).

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Dhar et al. (2006) analysed ethanolic extract of dried roots and leaves for withanolides and withaferin-A by HPLC. Identification of withaferin-A was done by TLC, UV absorption, HPLC and electron spray mass spectroscopy from suspension cultures of *W. somnifera* by Ciddi (2006). Five new phytoconstituents were isolated and their structures were elucidated from IR, NMR and mass spectrum by hydroalcoholic extract of *Hibiscus rosasinensis* and the hypotensive activity of these isolated compounds was also studied by Siddiqui et al. (2006). Alcoholic and aqueous extracts of *Nyctanthes abor-tristis* protected the liver from the toxic effect of CCl₄ by reducing the elevated levels of serum glutamate pyruvate transaminase, serum bilirubin at a dose of 500 mg/kg body weight of rat (Hukkeri et al., 2006).

Hepatoprotective effect of the alcoholic extract of *Centella asiatica* with oral administration (20 and 40 mg/kg/day) in rats having CCl₄ induced liver injury was studied. The plant extract effectively inhibited the biochemical changes. The histopathological examination of liver corroborated well with the biochemical changes. Hepatic steatosis, hydropic degeneration and necrosis observed in the CCl₄ treated group were completely absent in the herbal extract administered group (Antony et al., 2006).

Machiah et al. (2006) observed that the glycoprotein from *W. somnifera*, inhibits hyaluronidase activity of the venoms of cobra. Investigation of the withanolide from *W. somnifera* regulated the gene expression activated by various carcinogens. The withanolides suppressed the activation induced by a variety of inflammatory and carcinogenic agents including tumor necrosis factor, interleukin 1-β doxorubicin and cigarette smoke condensate (Ichikawa et al., 2006). Treatment of *W. somnifera* root powder (500 / 1000 mg/kg body wt.) reduced the level of paw volume and serum lysosomal enzyme in monosodium urate crystal-induced gouty arthritic rats. Administration of plant extract showed potent analgesic and antipyretic effect with the absence of gastric damage at different dose levels and the arthritic symptoms were reverted back to normal. This indicates the suppressive effect of root powder on the gout arthritis of experimental animal (Rasool and Varalakshmi, 2006).
Withanolides, steroidal lactones were isolated from in vitro cultured plant tissues of *Withania somnifera*. Five selected withanolides viz., withanone, withaferin-A, withanolide-A, withanolide-B and withanolide-E were identified by HPLC-UV (DAD) - positive ion electrospray ionization mass spectroscopy (Sharada *et al.*, 2007). Plant tissue culture experiments suggested that production of withanolides is closely associated with morphological differentiation. Kumar and Kalonia (2007) investigated the protective effect of *W. somnifera* in sleep disturbed mice. Treatment with *W. somnifera* extract and diazepam significantly reduced the anxiety levels in animals and improved locomotor activity. Similarly, biochemical studies showed a significant decrease in lipid peroxidation glutathione levels and improved catalase activity. Preliminary results suggested that the root extract can be used in the management of sleep loss associated oxidative stress. Sumantran *et al.* (2007) analysed the two novel condroprotective activities of aqueous extracts of *W. somnifera* root powder on damaged human osteoarthritic cartilage matrix. The extracts caused a significant and reproducible inhibition of the gelatinase activity of collagenase type 2 enzymes in vitro.

Visavadiya and Narasimhacharya (2007) investigated the hypocholesteremic and antioxidant effects of *W. somnifera* root powder in male albino rats. Administration of root powder (0.75 and 1.5 gm/rat/day) in the diet of the hypercholesteremic animals registered significant decreases in total lipids, cholesterol, and triglycerides in plasma and further a significant decrease in lipid-peroxidation occurred in *W. somnifera* administered hypercholesteremic animals when compared to their normal counterparts. Anandh Babu *et al.* (2007) evaluated the effect of *W. somnifera* Ethanolic extract on glucose-mediated collagen glycation (a diabetic complication and age related disease) and cross-linking in vitro. The activity of Ethanolic extract of *W. somnifera* is comparable to metformin, a known antiglycating agent and it could be have therapeutic role in the prevention of glycation induced pathogenesis in diabetes mellitus and aging. Diuretic activity of an infusion and a methanol extract of *W. somnifera* were studied in laboratory rats. Both infusion and methanol extract showed a significant diuretic effect compared with non-treated
controls with notable increases in water and sodium excretion. The potassium retention effect was also noticed (Martin-Herrera et al., 2007).

Oral administration of withaferin-A (20 mg/kg b.w) along with DMBA (7, 12-dimethyl benzanthracene a carcinogen which can induce oral cancer) for 14 week, completely prevented the tumour incidence in golden hamster. This shows its anti-lipid peroxidative and antioxidant properties (Manoharan et al., 2009). Treatment of ammonium chloride showed a significant increase in the levels of circulatory ammonia, urea, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), thiobarbituric acid and reactive substances (TBARS) and hydroperoxides (HP) in experimental rats. These changes were significantly decreased in rats treated with *W. somnifera* root powder and ammonium chloride which indicates *W. somnifera* offers hepatoprotection by influencing the levels of lipid peroxidation by the presence of alkaloids, withanolides, flavonoids etc. (Harikrishnan et al., 2008). Withaferin-A was found to induce apoptosis against several human leukemic cell lines from the patients having diseases like limphoblastics and myeloid leukemia (Mandal et al., 2008).

The combined effect of *W. coagulans* and *Trigonella foenum-graecum* exhibited hypoglycaemic activity in clinical patients. Significant improvement in symptoms and signs were observed. At the end of treatment a significant euglycemia was attained (Alam et al., 2009). Mahdi et al. (2009) studied the role of stress in male fertility and the ability of *W. somnifera* to combat stress induced male infertility. Administration of root powder at a rate of 5 g/day for 3 months to test patients, decreased the stress, improved the level of antioxidants and overall semen quality in a significant number of individuals.

### 2.3. Antimicrobial activity

The potential of higher plants as source of new antibiotic drugs is still largely unexplored. Medicinal plants represent a rich source of antibacterial agents (Mahesh and Sathish, 2008). In the present study, a systematic investigation was made to
evaluate the antibacterial activity of different solvent extracts of *W. somnifera*. A thorough survey on the antimicrobial properties of *W. somnifera* and some other important herbal plants are given below.

Antibacterial activity of crude alcoholic extract of *Datura alba* and *Celosia argentina* leaves was studied against pathogens isolated from infected burn patients. On comparing the efficiency of two extracts, the extract of *D. alba* exhibited more than 50% increase in antibacterial activity compared with *C. argentina*. The conventional antibiotic cream silver sulphadiazine (SSD) was used as standard (Gnanamani *et al.*, 2003). *In vitro* antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* was investigated against 143 laboratory strains belonging to 56 bacterial species, and 31 isolates of fungi using disc-diffusion assay. The methanolic extract had maximum inhibitory effects on the growth of 57 strains of 24 bacterial species in the genera of *Acinetobacter*, *Bacillus*, *Brevundimonas*, *Brucella*, *Staphylococcus* and *Xanthomonas* (Karaman *et al.*, 2003).

Antibacterial activity against six pathogenic strains was studied with aqueous, ethanol and chloroform extracts of *Alangium salviifolium* using disc diffusion method. Ethanolic extract showed the high degree of inhibition when compared with chloroform and aqueous extracts. The antibiotic chloromphenicol was compared as standard (Natarajan *et al.*, 2003). The methanol, hexane and diethyl ether extracts from both leaves and roots of *W. somnifera* were evaluated for its antibacterial activity by agar plate disc-diffusion against *Salmonella typhimurium* and *E. coli*. The minimum inhibitory concentration was 0.1 mg/l for *S. typhimurium* and *E. coli*. Among 6 extracts tested, only methanol and hexane extracts of both leaves and roots were found to have potent antibacterial activity (Arora *et al.*, 2004).

Petroleum ether, chloroform and methanolic leaf extracts of *Blumea balsamifera* were tested for the antibacterial activity against 12 broad spectrum human pathogenic bacteria. The plant extracts (25 mg/l) showed comparatively higher activity against a number of bacteria than the standard gentamicin (50 µg/ml). The results were
significant with P < 0.05 (Nessa et al., 2004). Njoku et al. (2004) investigated the antimicrobial activity of *Penisetum purpureum* against the pathogenic bacteria like *Bacillus subtilis, E. coli, Pseudomonas aeruginosa, Staphylococcus aureus* and the fungi *Aspergillus niger*. From his studies he observed a remarkable inhibition against all the tested organisms. Antibacterial activity of hexane, methanol and water extracts of leaf, stem and roots of *Clitoria ternatea* against gram positive and gram negative species of pathogenic bacteria was studied using minimal inhibitory assay. Methanolic extracts showed the highest activity and no activity was recorded with water extracts. Hexane and methanolic extracts showed a higher and significant antibacterial activity against both gram-positive and gram-negative bacteria. No antibacterial activity was recorded with *C. ternatea* stem extracts (Malabadi et al., 2005).

Screening of antimicrobial activity of *Tridax procumbens* using benzene and alcohol extracts of root, stem and leaves against *Salmonella typhi, Escherichia coli, Bacillus cereus, Shigella dysenteriae* and *Pseudomonas* species was done. All the extracts of root, stem and leaves showed inhibition to *Pseudomonas* sp. and *Shigella dysenteriae*, moderate and less activity against *S. typhi, E. coli* and *B. cereus* (Udayakumar et al., 2005). Antimicrobial activity of *Decalepsis hamiltonii* was assessed against seven human pathogenic microorganism viz., *Staphylococcus aureus, Yersinia enterocolitica, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, S. typhimurium* and *Candida albicans*. The herbal extract of *D. hamiltonii* root extract was most effective against *S. typhi, P. aeruginosa* and *E. coli* forming 20 mm zone of inhibition to each at 4 µg concentration. These results indicated that *D. hamiltonii* was a potent antimicrobial agent (Elizabeth et al., 2005).

Antibacterial activity of the rhizome oil of *Alpinia abundiflora* was tested against 10 strains of gram-positive and gram-negative bacteria in 3 dilutions, (1/10, 1/20, 1/30) using dimethylsulphoxide as the solvent. Comparison of the activity with streptomycin, shows that the oil is moderately active against the tested microorganisms in 1/10 dilution (Gopanraj et al., 2006). Different solvent extracts of *Convolvulus pluricaulis* were screened for their antibacterial efficacy against different
bacterial strains using disc diffusion method. The chloroform extract was found to be the most effective whereas the butanol fraction showed specific activity against *Staphylococcus epidermidis*. The effects were compared with those of ampicillin, streptomycin and tetracycline (Singh et al., 2006). Antibacterial activity of the crude extract from the leaves of *Aristolochia bracteata* with petroleum ether, chloroform and alcohol was studied against *Bacillus subtilis, Lactobacillus plantarum, Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa*. Among the three extracts used, the alcoholic extract showed significant antibacterial activity as compared to that of other extracts (Manikandar et al., 2006).

The antibacterial activities of 100 extracts of 50 Indian plant species were tested against six medicinally important bacterial strains viz., *Bacillus cereus, Staphylococcus aureus, S. epidermidis, Klebsiella pneumoniae, Alcaligenes faecalis* and *Pseudomonas aeruginosa*. The antibacterial assay was done by both agar disc diffusion method and agar well-diffusion method. The antibacterial activity exhibited by alcoholic extract was better than the aqueous extract. The results, evaluated as the diameter of the inhibition zone of microbial growth, showed that the extracts were more active against gram-positive bacteria than the gram-negative bacteria. The maximum activity was shown by *Terminalia chebula, Mangifera indica* and *Eucalyptus citriodora* (Parekh and Chanda, 2006).

Screening of antibacterial potentiality from aqueous and methanolic extract of 12 plants viz., *Abutilon indicum, Acorus calamus, Ammania baccifera, Argyrea nervosa, Bauhinia variegata, Crataeva religiosa, Hedychium spicatum, Holarrhena antidysentrica, Piper nigrum, Plumbago zeylanica, Psoralea corylifolia, Saussurea lappa* was studied against five bacterial strains viz., *Bacillus cereus, Staphylococcus aureus, Klebsiella pneumoniae, K. pseudoalcaligens, Escherichia coli* and *Pseudomonas sp.* using disc diffusion and well diffusion method. The preliminary experiment revealed that the methanol extracts of all the plants were more potent than the aqueous extract. The methanolic extract were more effective against gram-positive than the gram-negative bacteria. Among all the plant species studied, *Bauhinia*
variegate exhibited remarkable antibacterial activity (Parekh and Chanda, 2006). The antibacterial activity of Hybanthus enneaspermus was investigated against 6 urinary infections causing bacterial pathogens viz., E. coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis, Enterococcus faecalis and Staphylococcus aureus by disc diffusion assay method. Ethanol extract exhibited significant and broader spectrum of inhibition (MIC) compared with aqueous, chloroform and petroleum extract (Sahoo et al., 2006).

The methanolic leaf extract of W. somnifera, Acacia nilotica, Sida cordifolia, Tinospora cardifolia and Ziziphus mauritiana showed significant antibacterial activity against Bacillus subtilis, Escherichia coli, Pseudomonas fluorescens, Staphylococcus aureus and Xanthomonas axonopodis (Mahesh and Satish, 2008). Leaf extract showed significant activity when compared with the bark and root extract of all the test plant extract. In vitro antibacterial activity of aqueous and methanol extracts of Acacia holosericea, Ipomea carnea, Justicia gendurussa and W. somnifera were screened against multi-drug resistant bacteria including Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis and Streptococcus pyogens isolated from clinical specimens. Methanolic extracts of W. somnifera showed higher antibacterial activity compared to other plant extracts tested (Rajendran and Ramakrishnan, 2009).

2.4. Plant Tissue culture
2.4.1. Withania somnifera

Multiple shoots were induced from the shoot tip explant of W. somnifera on MS medium containing BAP (8.9 μmol) along with 2, 4-D (2.3 μmol) or IBA (2.5 μmol). Direct shoot multiplication was also obtained from germinating seeds in the presence of BAP (4.4 μm) alone. Rooting was successful in excised shoots grown on growth regulator free medium (Sen and Sharma, 1991). An efficient protocol was standardized for in vitro propagation of W. somnifera from leaf explants of in vitro grown two month old seedlings. IAA and BAP induced direct shoots. The shoots were elongated and rooted on MS medium containing BAP (0.04 μmol). By this method
1600 plantlets could be produced from a single leaf (Kulkarni et al., 1996). Callus cultures were initiated from axillary leaves, axillary shoots, hypocotyl and root segments of *W. somnifera* on MS medium supplemented with 2,4-D (2 mg/l) and KN (0.2 mg/l). Best shoot differentiation was observed from axillary shoot base callus on BAP (2 mg/l) containing media, and shoots were rooted by IBA (2 mg/l) alone or with IAA (2 mg/l) (Gita Rani and Grower, 1999).

Direct regeneration of shoot buds was observed in MS medium supplemented with BAP (0.1 to 5.0 mg/l) from nodal, internodal and hypocotyls explants. TDZ was also equally performed. Shoot buds elongated and rooted with low concentration of BA (0.01 mg/l) or on half strength medium lacking growth regulators (Anjali Kulkarni et al., 2000). Friable soft callus was obtained from stem explants on MS medium supplemented with 2.2 \( \mu \)mol 2, 4-D. The maximum shoot regeneration was achieved in 4 week old, white friable callus on MS medium fortified with 4.4 \( \mu \)mol BA and 0.5 \( \mu \)mol IAA. The isolated shoots were multiplied on the medium containing BAP (4.4 \( \mu \)mol) alone. Regenerated shoots were rooted on half strength MS medium supplemented with 9.4 \( \mu \)mol IBA (Manickam et al., 2000).

Callus induction was observed from hypocotyl segments grown on MS medium supplemented with the combination of 2, 4-D (2 mg/l) and KN (0.2 mg/l). When this callus were subcultured on the same medium, regenerated numerous shoots. These shoots were transferred to MS medium containing BAP (2.5 mg/l) or 2ip (2 mg/l), but maximum shoot multiplication was noticed with BAP. Shoots were rooted best on MS medium containing 2 mg/l of IBA (Gita Rani et al., 2003). Callus cultures initiated from nodal segments on MS medium containing BAP (1 mg/l), KN (2 mg/l) was found organogenic. Such callus when subcultured on MS medium containing BAP (1 mg/l) and KN (2 mg/l) differentiated adventitious shoots. Regenerated shoots rooted best on MS medium containing IBA and KN (each 1 mg/l) (Siddique et al., 2004).

A high frequency and rapid regeneration protocol via callus and directly from various explants was developed. Regeneration was observed from the callus of all
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epoxlants except roots on MS medium fortified with BAP (0.5 to 2.5 mg/l) alone or along with IAA (0.5 mg/l). Direct regeneration was noticed on MS medium supplemented with BAP and low concentration of IAA. Dwarf shoots were elongated on MS medium with GA₃ (0.5 mg/l). Plantlets were rooted in half strength MS medium (liquid or solid) with IBA (1 mg/l) alone or along with IAA (0.5 mg/l) (Govindaraju et al., 2003). Axillary bud multiplication and indirect organogenesis were achieved from nodal explants on MS medium with a combination of BAP (3.3 μmol), KN (1.16 μmol) and IBA (0.98 μmol). Callus from basal cut ends of the explants differentiated more number of shoots on the MS medium with BAP (4.4 μmol) and IBA (0.98 μmol). About 90% of rooting was achieved on MS medium with IBA (3.6 μmol) (Ashutosh et al., 2004).

The morphogenetic potential of apical and nodal bud and embryos was studied by Kannan et al. (2005). They observed shoot regeneration from embryo through callus on the medium containing high ratio of cytokinin-axin (BAP/KN and NAA). Medium with BAP 10 μmol and NAA 10 μmol induced profuse budding from both shoot tip and node. Rooting capacity of regenerated shoots was compared with NAA, IBA and IAA (1.5 mg/l). NAA produced roots within a short period at lower concentration (2 μmol). Higher level of IBA (10 μmol) was found essential for the induction of normal roots.

Sivanesan (2007) compared different media (MS, SH and B5) for the shoot multiplication from the shoot tip explants of mature plants of W. somnifera. MS medium was found superior to SH and B5 medium. In MS medium, BAP and IAA (each 2 mg/l) was optimal for shoot induction, GA₃ (0.3 mg/l) was more suitable for shoot elongation. Elongated shoots were rooted on half strength MS medium containing with 2 mg/l of IBA. MS medium supplemented with BAP (0.6 mg/l) with IAA (0.4 mg/l) was found to be most effective in initiating multiple shoots at the rate of ten per explant in W. somnifera. By repeated subcultures, high frequency of shoot multiplication was established. About 90% of rooting was achieved in combination with 0.4 mg/l IBA and 0.4 mg/l IAA. Micropropagated plants were hardened in half
strength MS medium and then established in sand and soil (1:1) mixture (Ujjwala Supe et al., 2006). MS medium supplemented with BA (2 mg/l) and NAA (0.1 mg/l) was found optimum for the production of multiple shoots from shoot tip explants. The regenerated shoots were found to produce flower buds after 6 weeks of culture in the medium supplemented with KN (0.5 to 4 mg/l) and IAA (0.1 mg/l). *In vitro* fruiting was also observed in the presence of KN (2 mg/l) and IAA (0.1 mg/l) in *W. somnifera* (Saritha and Naidu, 2007).

Shoots were induced from axillary buds of *W. somnifera* on MS medium supplemented with BAP (0.25 mg/l) along with GA₃. The regenerated shoots were rooted on MS medium supplemented with NAA (0.5 mg/l) along with GA₃ (Sharma et al., 2009). Maximum number of shoots in all accessions was achieved from axillary explants of *W. somnifera* on MS medium supplemented with BAP (1.0 mg/l) and KN (1.0 mg/l). Inclusion of KN, increased shoot numbers in a shorter period and it was effective on all the elite accessions. Highest number of shoots 60 ± 1.82 in BAP and 60.05 ± 2.03 in KN was observed. Rooting was high in 2 mg/l IBA. Rooted shoots were hardened successfully in garden soil – vermicompost (3:1 w/w) under a glasshouse (Sabir et al., 2008).

2.4.2. Other plant species

Nodal segments produced multiple shoots while apical meristems and cladodes proliferated into callus on MS medium supplemented with NAA (0.1 mg/l) and KN (2 mg/l). Kinetin favoured the multiple shoot induction in 80% cultures while the increased amount of NAA promoted the callus induction in *Asparagus racemosus* (Neetu vijay and Ashwani kumar, 2004). Direct shoot development without any intervening callus phase was observed when the leaf explants were cultured on MS medium supplemented either with BAP alone or in combination with IAA or NAA in *Physalis peruviana*. The auxins 2,4-D, 2,4,5T, IAA or NAA produced only roots from the explants. Maximum percentage of regeneration and more number of adventitious shoots were obtained with IAA (2.85 µM/l) and BAP (13.20 µM). Highest rooting on leaf explants was observed on MS media supplemented with 2, 4, 5-T (7.8 µM/l) or
2,4-D (4.52 μM/l). Rooting of regenerated shoots was occurred on hormone free MS medium (Jayasree et al., 2005).

Culture of nodal explants with BAP (0.3 mg/l) and KN (0.2 mg/l) produced 1-4 axillary shoots in Centella asiatica. Combination of BAP (3.0 mg/l) either alone or in combination with IBA (0.5 mg/l) was found most effective for axillary bud multiplication (George et al., 2004). On MS medium with a combination of 2, 4-D (2 mg/l) and KN (0.2 mg/l), 100% of callus induction was obtained from root and leaf segments of C. asiatica. Maximum shoot multiplication was observed after 60 days of the second subculture of callus on the medium containing BAP (2 mg/l) (Rani et al., 2003). The optimum number of shoots (3.38) with optimum number of leaves per shoot (4.25) was obtained on MS medium supplemented with BAP (4.0 mg/l) and NAA (0.1 mg/l). The shoots were transferred with various concentrations of IBA (1.0 – 3.0 mg/l) and NAA (0.5 to 2.0 mg/l). Profuse rooting (46.8 per shoot) was induced by IBA (2.0 mg/l) with a root length of 19.7 cm in Adhatoda vasica (Sangeetha and Buragohain, 2005).

High frequency of shoot regeneration from shoot tip, nodal and internodal segments of Phyllanthus amarus was observed on MS medium supplemented with different concentrations of BAP and KN with 15% coconut milk. High frequency of (100%) regeneration and maximum number of shoots (18.30 ± 0.47) were recorded on medium containing BAP (0.5 mg/l) from shoot tip culture. The shoot clumps were transferred to rooting medium containing different concentrations of IBA and NAA. Maximum number of root was induced in IBA at 0.5 mg/l level. Rooted plants were hardened on MS basal liquid medium added sterile soil + vermiculite (1:1). The survival rate of plantlets in the field was found to be very high (85%) (Kiran Ghanti et al., 2004).

An efficient micropropagation method was developed from apical and axillary bud explants of Heliotropium indicum. Highest number of shoots (32.6 shoots in apical bud and 20.2 shoots in axillary bud) was recorded after 30 days of culture on
MS medium supplemented with KN (1.0 mg/l), BAP (0.5 mg/l) and IAA (0.05 mg/l) and the shoots were elongated on the same medium. Highest frequency of rooting (85%) was obtained in both apical and axillary bud derived shoots in half strength MS medium supplemented with IBA (0.1 mg/l) (Senthilkumar and Rao, 2007). Tejavathi et al. (2010) observed the regeneration of multiple shoots from cotyledonary node derived callus of *Macrotyloma uniflorum*. MS medium supplemented with NAA (1.86 μM), BAP (0.45 μM) and IBA (0.45 μM), BAP (0.11 μM) results 14 and 20 shoots from callus after 30 days of culture. The regenerated shoots were rooted on MS medium with IAA (0.11 μM), KN (0.21 μM) results 6 roots per shoot. A maximum of 60% survival rate was noticed on a mixture of soil, sand and manure (1:1:1 ratio).

2.5. Transformation

Recent approach to improve medicinal plants is genetic transformation. The rapid progress in the area of medicinal plant biotechnology is mainly because of the development of efficient regeneration and suitable *Agrobacterium* mediated transformation protocols. Successful report on genetic manipulation of ashwagandha and some other important herbal plant were collected and presented here.

Transformation of *Agrobacterium tumefaciens* in *Triticum aestivum* resulted very low frequencies of DNA transfer by Mooney *et al.* (1991). Transformation of Paenax ginseng was achieved by means of *A. tumefaciens* strain LBA 4404 harbouring the binary vector PB1121 in MS medium with 2, 4-D (1.0 mg/l) and KN (1.0 mg/l) and Kanamycin 100 mg/l. Callus formed after 8 weeks in the cut surface of the explants showed *gus* expression to confirm the transformation (78%) (Lee *et al*., 1995). *Agrobacterium* mediated tumour tissue and shooty teratomas of *Coleus forskohlii* were cultured *in vitro*. The active principle forskolin was detected in tumorous callus (0.002%) rhizogenic callus (0.011%) and root cultures (0.014%) but not in shooty teratomas (Swapna *et al*., 1996).

Transformation of *Chichorium intybus* shoot buds was obtained by *A. tumefaciens* containing binary plasmids *nptII* and GUS. The transformation
frequency was 10% after 4 weeks of co-cultivation on of kanamycin (100 mg/l) selection medium (Fanny Frulleux et al., 1997). Hairy root cultures were obtained from plantlets co-cultivated with a virulent, *A. rhizogenes* and disarmed *A. tumefaciens* strains in grape wine. The transformation frequencies of hairy root clones ranged from 4 to 16% in Kanamycin selection medium (Torregrosa and Bouquot, 1997). Transformation of *A. tumefaciens* was carried out in stem, leaf and microtuber disc explants of potato (Yang and Zhou, 1997).

Transgenic herbicide-resistant plants were obtained by co-cultivation of leaf explants with *A. rhizogenes* from *Antirrhinum majus*. The hairy roots were induced from leaf explants on 1 mg/l bialaphos-containing half-strength MS medium (Hoshino et al., 1998). Withanolide synthesis in shooty teratomas was much higher in the amount of withaferin-A (0.07 – 0.1%) and withanolide-D (0.085 – 0.025%) than in non-transformed shoot cultures. The transformation experiment was established by the infection of wild type nopaline and octopine strains of *A. tumefaciens* in MS basal medium (Ray and Jha, 1999).

Hairy roots of *Panax ginseng* were obtained from root discs explants using a wild strain of *A. rhizogenes* 15834. The hairy root formation was 5-20 in number per explants but the transformation frequency was very low (1%) (Yang and Choi, 2000). Stable transformation and expression of transgene was achieved in *Chicorium intybus* using *A. rhizogenes* mediated system. The transformation frequencies varied with the use of different types of *A. rhizogenes* strains and the age of explants. Hairy roots were induced from ginseng roots by using *A. rhizogenes* on MS medium without phytohormones. The induced roots were dense branching, clustering and non-geotropic (Shoujing et al., 2001).

*Agrobacterium* mediated transformation system was developed for two varieties of *Lens culinaris*. The transformation ability was tested in cotyledonary node, decapitated embryo, immature embryo, and epicotyls with the *A. tumefaciens* strain LBA 4404 harbouring binary vector PB1121 containing GUS and nptII genes. Highest
GUS performance was found in epicotyls and selection of transformation was carried out by gradually increasing the concentration of kanamycin 200 mg/l in MS medium supplemented with 0.5 mg/l BAP, 0.5 mg/l KN, 0.1 mg/l GA₃ and 5.5 mg/l tyrosine (Sarker et al., 2003). Genetic transformation of *Bacopa monnieri* was standardized using the *A. tumefaciens* strain EHA 105 harboured the binary vector pBE 2113 containing GUS and neomycin phosphotransferase. The leaf explants produced kanamycin resistant calluses and resistant plantlets and were confirmed by GUS activity (Nisha et al., 2003).

*Agrobacterium rhizogenes* mediated hairy root induction was investigated in *W. somnifera* and *Solanum surattense* using stem, hypocotyl and leaf (with midrib) explants by *in vitro*. MS basal medium with exogeneous supply of phytohormones was used. The rate of transformed roots observed was 10 fold as compared with control (Pawar and Maheswari, 2003). Four highly branched root clones were obtained from leaf explants of bisexual *Actinidia kolomita* plants by *A. rhizogenes* mediated transformation. Transformed shoots were regenerated from these roots on half strength woody plant medium with BAP (0.5 to 5.0 mg/l). Then the transformed micro cuttings were formed dense roots on auxine free MS medium (Vardja and Vardja, 2004). Hairy root induction was performed using wild type strain of *A. rhizogenes*. A4 LBA 9402 and 2659 on seven different leguminous plants viz., *Arachis hypogea, Cajanus cajan, Cicer arietinum, Glycine max, Vigna aconitifolia, V. mungo* and *V. radiata*. The frequency of response was varied with the plant species. Hairy roots and multiple shoots were developed on MS medium supplemented with cefotaxime (Kamle and Eapen, 2005).

*Agrobacterium tumefaciens* mediated genetic transformations were studied by using binary vector p35 SGUSINT on 6 days old seedlings of *Arachis hypogea*. The cocultivated explants showed 45% success in kanamycin resistance and the transformation frequency was 31% by GUS assay (Swathi et al., 2006). Direct inoculation using with wild type *A. rhizogenes* to the *in vitro* stem and leaf tissues of *Gentiana triflora* and *G. scabra*, induced a number of hairy roots on MS medium.
containing NAA (3 mg/l) and TDZ (10 mg/l) (Mishiba et al., 2006). An efficient and reproducible method for *Agrobacterium* mediated transformation of mature embryo of *Triticum aestivum* and *T. durum* was standardised in the presence of 200 μM acetosyringone with bacterial medium. The transformation frequency was ranging from 1.28 to 1.77% on 2-3 days of co-cultivation (Patnaik et al., 2006).

A transformation protocol was developed for rapid and efficient production of transgenic plants *via* somatic embryo and regeneration from the CVS XP116 leaf, cotyledons and hypocotyl explants of *Apium graveolens*. GUS expression confirmed the transformation and the transformation frequency was 5.0% and 5.0% for leaf, 17.8% and 18.3% for cotyledons and 15.9% and 16.7% for hypocotyls explants (Guo-Quinsong et al., 2006). *A. rhizogenes* mediated transformation was studied from 2 to 5 weeks old *in vitro* grown leaf explants of *Glycyrrhiza glabra*. The strain K599 was used to infect the explants on MS medium. The leaf explants of 2 and 5 weeks old cultures were not responsive, 3 and 4 weeks old explants could induce hairy roots. The transformation frequency was 47% obtained in 3 weeks old explants after 25 days of incubation (Mehrotra et al., 2008). Inoculation of *W. somnifera* with *A. rhizogenes* strain LBA 9402 and A4 produced typical transformed root lines, transformed callus lines and rooty callus lines with simultaneous differentiation and redifferentiation. The morphologically distinct transformed lines varied in T-DNA content, growth rates, and withanosteroid accumulation (Bandyopadhyay et al., 2007). Transformation studies of four potato cultivar were performed using the *A. tumefaciens* harbouring β-glucurinidase (GUS) gene. The highest percentage of shoot formation obtained in stem explants (91.6%) and maximum GUS positive expression was 92.8% in leaf explants (Badr et al., 2008).

The natural transformation with genes encoding the production of microbial elicitors could influence the interactions between plants and other organisms. Thus induce the pathogen defense response and increase the metabolite and biomass production in transformed root cultures of *W. somnifera* (Kuntal chaudhuri et al., 2009). Genetic transformation method was developed using *A. tumefaciens* strain LBA
4404 containing the PCAMBIA 2301 plasmid harbouring nptII and GUS in the T-DNA region in the presence of 200 μM acetosyringone was developed in Phyllanthus amarus. The transformation efficiency was found to be 54.6% and it was confirmed by kanamycin-resistance (Anindita and Sharmila, 2009). A. tumefaciens mediated transformation was performed using the strain LBA 4404 containing the binary vector PLG 121 Hm along with gus A reporter gene with intron in the leaf segments of W. somnifera. Selection of transgenic shoots was done in the presence of 50 mg/l kanamycin. Maximum transformation efficiency was found to be 1.67% (Pandey et al., 2010).

In the light of these, it is worthwhile to undertake in vitro studies on W. somnifera with a view to develop reproducible protocol for mass propagation, genetic improvement and to assess the nephroprotective activity of this valuable medicinal plant.